

## PART V

### *Methods*

## I. THE PREPARATION OF ATP\*

Research on muscular contraction is greatly dependent on a generous supply of ATP. The methods hitherto employed are rather expensive and their yield is low. In these methods the tissue is usually extracted with trichloroacetic acid which precipitates the proteins and extracts the ATP which is then isolated as the Hg, Ca, or Ba salt.

The method to be described, worked out by J. Banga and myself and somewhat modified later by W. Sz. Hermann, is based on the observation that ATP, in muscle, is adsorbed to the structure and is precipitated with it if anhydrous solvents (alcohol) are used. The denatured protein releases the ATP on extraction with water. In this way the ATP is obtained in relatively high concentration and purity, the major part of soluble substances having been removed by the alcohol. This method allowed us to prepare about 1 kg. of ATP, used in the research described in this book. The method is cheap, the alcohol being recovered with little loss.

The procedure is the following:

The animal (rabbit or horse) is killed. Its muscles are rapidly minced and dropped from the mincer into 96% alcohol, 4-5 liters of 96% ethyl alcohol being used for every kg. of meat. After one or two hours of occasional stirring, the muscle residue is separated from the alcohol either on a press or by centrifugation. The muscle residue is suspended again in alcohol, one liter being used for every kg. of muscle. The suspension is brought to boiling, allowed to cool, and the alcohol again separated. This second alcohol extracts some more soluble matter and makes the protein quite insoluble. The muscle residue is dried in air and reduced to a powder in a mill. The dry muscle can be stored in a dry place indefinitely.

The powdered muscle is extracted with water. At the beginning, we extracted 1 kg. of powder with five liters of water

\*The industrial use of the method is covered by patents both in Europe and the Americas.

for ten minutes, pressed out the water, suspended the muscle in three liters of water, pressed again after ten minutes, repeating this last procedure a second time. In this way about ten liters of extract was obtained.

Later it was found more convenient to separate the muscle on a centrifuge, a type being used which is found in laundries. By this method the volume of the extract could be reduced to six liters, three liters being used for the first and one to two liters for the second and third extractions.

To the six liters of fluid obtained on extracting one kg. of muscle-powder, four ml. of glacial acetic acid was added per liter. The precipitate formed carried down the glycogen present and was removed. Then 200–250 ml. of 20% mercuric acetate was added (dissolved in 2% acetic acid) depending on the quantity of ATP present. After a short sedimentation the precipitate was separated on the centrifuge, the inorganic phosphates being left in solution. The loss in this precipitation amounted to 10%.

The precipitate was suspended in 250 ml. 0° 0.5 M HCl and decomposed with  $\text{H}_2\text{S}$  at 0°. It was separated on the centrifuge in the cold, washed with 50 ml. of 0.5 N cold HCl, and centrifuged again. The extracts were combined, treated once more with  $\text{H}_2\text{S}$ , and centrifuged. The  $\text{H}_2\text{S}$  was eliminated by aeration. Then 100 ml. of 10%  $\text{CaCl}_2$  was added (prepared by dissolving 10%  $\text{CaCl}_2$  in 96% alcohol). Four vols. of alcohol were added and the fluid kept overnight in the icebox. The precipitate was separated, washed with alcohol until free of Cl, and dried; it contained the ATP in the form of its acid Ca-salt, with one Ca per mol ATP.

About half of the ATP present in muscle (2.5 mg. per g. muscle) was obtained this way in high (about 90%) purity. Further purification could be effected by standard methods.

The ATP was liberated for experimental use in the following way: 1 g. Ca salt was dissolved in 30 ml. water, then 10% K oxalate was added until the fluid contained neither Ca nor excess oxalate. Then the fluid was neutralized with KOH, made up to 60 ml., and centrifuged. One ml. contained about 12 mg. ATP.

## II. THE PREPARATION OF MYOSIN\*

*General Remarks*

Myosin is very sensitive to the action of heavy metals: it strongly binds and accumulates them, undergoing denaturation. Working with myosin, metal-free water must be used, distilled from glass vessels through glass coolers. The salts used for the extraction of myosin in high concentration must be of high purity. KCl has been preferred in this work as the neutral salt, but its action is not specific.

Some properties of myosin are very labile, thus its extraction and preparation must be done at a low temperature (0°). Unfortunately, it is not possible to work throughout at this temperature because actomyosin is not precipitated by ATP at 0°. ATP has a stabilizing action, as do salts, in the absence of which myosin is more unstable.

*Extraction*

As described before, there are reasons to believe that in muscle both actin and myosin form a continuous system, and it is the actin system which unites with the myosin system to form actomyosin. To extract myosin, it must be separated from actin and the links connecting the myosin micels to a continuous system must be disrupted. To do this the myosin particle must be charged and hydrated. To reach the necessary degree of charge and hydration, the simultaneous action of ATP and of a high concentration of salts is needed. ATP is present in fresh muscle in a sufficiently high concentration, but if the muscle is stored the ATP is decomposed and the myosin becomes insoluble. If myosin is kept in the absence of ATP at a lower salt concentration, cohesive links develop which make dissolution increasingly difficult.

To bring myosin of muscle into solution, KCl must be present in at least 0.5 M concentration. Once the myosin was dissolved and the myosin system broken up into smaller units, it is soluble even in 0.1 M KCl.

\*Crystalline myosin was prepared routinely by this method in the author's laboratory for a long period, with very few failures.

To precipitate myosin from its solution, the salt must be diluted strongly; thus any excess of salt during extraction involves additional dilution and therewith loss of material. The solvating action of ATP is greatly enhanced by the Mg and the proteins adsorbed to myosin. The separation of dissolved actin from the dissolved myosin involves a rather heavy loss of myosin. Hence, if crystalline, actin-free myosin is needed, it should, if possible, be free of actin in the first extract.

Increased pH favors the dissolution of actin. The solubility of actin varies also in different animal species. The rabbit, as shown by Guba (70, page 56) is an especially favorable material for the preparation of pure myosin. The experiments reported in this book relate to this material.\*

The animals were killed by decapitation, rapidly skinned, eviscerated, and dipped into ice water for 2-3 minutes. Then the muscles were excised and covered with minced ice. The muscles were treated 2-3 minutes later in a mincer with a sieve plate having holes of 2 mm. diameter. The mince was suspended at once in the ice-cold salt solution.

The dissolutions of myosin and actin follow different time curves. Myosin dissolves rather rapidly, while actin dissolves slowly; therefore, the extraction should be of short duration.

The alkaline KCl of Weber,† used by most previous research workers, yields a myosin rather heavily contaminated with actin. KCl alone (0.6 M) extracts myosin with varying actin content.

The purest myosin of fairly reproducible qualities could be obtained with the acid KCl-phosphate solution of Guba and Straub. This fluid contains 0.3 M KCl and 0.15 M K-phosphate of pH 6.5. Every 100 g. of the minced muscle were suspended in 300 ml. of this fluid of 0°. The mince was extracted for ten minutes under constant, gentle stirring. After this time the suspension was diluted with water of room temperature, four volumes being added for every volume of salt solution used. The suspension was then rapidly strained

\*Since glycogen also precipitates myosin, it is advisable to use rabbits which have fasted for 1-2 days.

†0.6 M KCl, 0.04 M NaHCO<sub>3</sub> and 0.01 M NaHCO<sub>3</sub>.

through a cloth and pressed out. The resulting turbid liquid was warmed to 20° and stirred very gently. The fluid now contained myosin, some actin, and ATP. At this ionic concentration, the ATP present keeps the actomyosin in dissociated condition. The ATP is gradually split by the myosin, and its concentration decreases within 1–2 hours to such an extent that it no longer dissociates the actomyosin but causes it to undergo a floccular precipitation. The precipitate consists mostly of actomyosin containing 1.5 parts of actin to 98.5 parts of myosin. The excess of myosin is left in solution. If the muscle extract contains more actin than 1.5 parts to 98.5 parts of myosin, no actin free myosin can be obtained by this method, the whole myosin being precipitated as actomyosin.\*

During the formation of the precipitate, the fluid must be stirred gently. If the stirring is omitted, the actomyosin forms a very fine colloidal precipitate which cannot be separated on centrifugation. The result is the same if the stirring is too violent.

#### *Crystallization*

The precipitated actomyosin is separated on the centrifuge at once and rejected. The fluid is rapidly cooled to 0°. The myosin is now, in the absence of ATP, rather labile. The fluid is strongly stirred and diluted with 1.5 volumes of ice-cold distilled water. The water is run in slowly, its addition being effected in about 10 minutes. The myosin precipitates in the form of fine needles. The silky sheen of the stirred fluid indicates crystallization. The strong stirring provides coaxial orientation of particles and favors crystallization.

The crystalline suspension is allowed to stand for one to two hours at 0°, decanted, and the crystals separated by centrifugation at 0°. In spite of its crystalline nature, the precipitate is very voluminous and usually contains no more than 5% of solid matter.

Muscle contains 8–10% myosin. About  $\frac{1}{3}$  of this quantity

\*The relative proportion of actin : myosin in the precipitate depends on the salt concentration. The smaller the ionic concentration, the lower the ratio in the precipitate.

is extracted by the phosphate; 50% is lost on crystallization, being left behind in the mother liquor.

#### *Further Purification*

The crystalline precipitate may be purified in various ways. It may be suspended in various volumes of 0.025 M KCl (the author usually used one liter if the crystals were obtained from 10 liters of fluid.)

The myosin may be recrystallized by dissolving it in 0.6 M KCl, powdered salt or 2 M KCl solution being added to the precipitate. The solution should contain no more than 3% myosin and should not be very viscous. The solution is diluted very gradually under strong stirring at 0° until the KCl concentration is decreased to 0.04–0.025 M.

The myosin at this stage still contains a small quantity of actin from which it cannot be liberated by recrystallization. Myosin may crystallize with as much as 3% actin, a quantity which may considerably influence the enzymatic reactions or physical properties of myosin.

To separate myosin from actin, it was dissolved by the gradual addition of 0.02 M  $K_2CO_3$ , containing 0.01% phenolphthalein. Carbonate was added until the solution retained a faint rose color (pH 8.3). Then, for every g. of myosin present, 4 ml. of 2 M KCl were added and the fluid homogenized by stirring. Then, for every ml. of KCl used, 50 ml. of water at room temperature (20°) were added under strong stirring. This water contained 0.001% phenolphthalein and a sufficient quantity of  $K_2CO_3$  to give it a faint rose color. If the rose color disappeared, it was re-established by the addition of carbonate-phenolphthalein mixture. The voluminous precipitate contained the actomyosin present and was separated on the centrifuge at room temperature. The faint rose, opalescent fluid was poured off and cooled. The precipitate still included considerable quantities of myosin; again 2 M KCl was added, this time half as much as before, followed by the addition of the corresponding quantity of water, and centrifugation. The united fluids were cooled to 0°, then 1% acetic acid was added very gradually with strong stirring until the pH fell to 7. The

myosin separated in the form of somewhat irregular needles, which showed no "activity" (no drop of viscosity on addition of ATP to the 0.5 M KCl solution).

The myosin obtained on the first crystallization, followed by washing with 0.025 M KCl, contracts energetically if converted into actomyosin by the addition of actin. It deaminates ATP and ADP and splits off both phosphates from ATP. The same holds for recrystallized myosin and most preparations of the actin-free myosin purified by the carbonate method.

#### *Ammonium Sulphate Method*

The carbonate method previously described for liberating myosin from actin is rather unsatisfactory, however valuable its services were. The manipulations are too complicated, involve a large loss of material, and expose myosin to denaturation. The development of another method was shown by M. Dubuisson\* who fractionated "myosin" by ammonium sulphate into two different fractions which he called  $\alpha$ - and  $\beta$ -myosin. There were reasons to believe that Dubuisson's  $\alpha$ -myosin was actomyosin, his  $\beta$ -myosin a rather actin-free myosin, actin or actomyosin being precipitated at a lower degree of  $(\text{NH}_4)_2\text{SO}_4$  saturation than myosin.† B. Horvath undertook the study of  $\alpha$ - and  $\beta$ -myosin and found that  $\alpha$ -myosin had all the properties of actomyosin while  $\beta$ -myosin was a rather actin-free myosin. The precipitability of actomyosin depends on its actin content. The higher the actin content, the lower the saturation at which the actomyosin precipitated.

Our experience with this method is not extensive enough to allow very accurate directions. The method found most convenient was to subject 1/2% myosin solution to the  $(\text{NH}_4)_2\text{SO}_4$  fractionation. The actomyosin being precipitated according to its actin content at 27–40% saturation, the solution is brought to 40% saturation at 0° (pH 6–6.5), the precipitate separated on the centrifuge, the fluid brought to 45–47% saturation, the myosin centrifuged out, dialyzed overnight at 0°, and precip-

\*M. Dubuisson. *Experientia*, 2, 413, 1946.

†Pure actin is precipitated at pH 5.5 at 20%, at pH 6–8 at 25–35% ammonium sulphate saturation.



itated by dilution. The myosin thus obtained is clearly soluble in 0.5 M KCl, is enzymatically very active, and contracts (with actin and ATP) most energetically. It seems to be very rich in protins.

Though our experience with this method is rather limited, certain of its advantages may be indicated. As mentioned before, there are reasons to believe that the myosin thus prepared is more actin-free than the myosin obtained with earlier methods. High salt concentrations dissociate actomyosin, and ammonium sulphate denatures actin,\* which might also contribute to rendering the myosin free of actin. Another evident advantage of the  $(\text{NH}_4)_2\text{SO}_4$  method is that it permits preparing actin-free myosin from material contaminated with actin rather heavily, which was not the case with our earlier method.

### III. THE SEPARATION OF PROTINS AND MYOSIN

#### *The Contraction Test*

The myosin is dissolved in 0.5 M KCl. A fibrous and protin-free actin solution is prepared. Myosin, KCl, and  $\text{MgCl}_2$  solution and water are pipetted into a small test tube in such a way that the 1.8 ml. fluid contains 2 mg. myosin, 0.05 M KCl, 0.001 M  $\text{MgCl}_2$ , and 2 mg. ATP or ADP. The test tube is shaken thoroughly and placed into a water bath at room temperature. Then 0.2 ml. 0.2% fibrous actin is added and the tube shaken thoroughly once. If a sufficient quantity of protin is present, contraction ensues within  $\frac{1}{2}$ -3 minutes. Contraction can best be observed in a water bath with flat glass walls, illuminated from behind through an opaque screen. The neutrality of the myosin, actin, and ATP or ADP solution provides the neutral reaction. Reactivation of inactive myosin preparations is effected by adding watery muscle extract to the myosin solution.

#### *Protin-free Myosin*

To prepare protin-free myosin for his ADP contraction test, Guba recrystallized his myosin prepared by the acid

\*Straub, unpublished.

phosphate method. The crystalline myosin precipitate formed on dilution was not separated on the centrifuge at once but was allowed to stand in both crystallizations at  $0^{\circ}$  with gentle stirring for six hours, time being given to the protin to become dissociated. The actomyosin prepared from this myosin did not contract on addition of ADP.

In preparing myosin for the ATP-contraction test, the "activity" (drop of viscosity on addition of ATP in 0.5 M KCl) was tested after the first crystallization. If the myosin showed any "activity," indicating the presence of actin, it was put through the carbonate purification (see preparation of myosin, page 138). After the second crystallization, the myosin was suspended in a large volume of 0.025 M KCl brought to pH 6 by 0.01 M acetate buffer. For every g. of myosin about 1 l. of fluid was taken. The suspension was stored at  $0^{\circ}$  with gentle stirring for 5–36 hours and tested repeatedly for contraction. The rate at which it became unable to contract depended on the quantity of actin present. The best results were obtained with myosin prepared by the ammonium sulphate method. In the case of myosin, showing a negative "activity" (rise of viscosity with ATP in 0.5 M KCl), and being thus probably completely free of actin, the protein could be deprived of its contractility within five hours.

Banga, to prepare myosin unable to dephosphorylate ADP, suspended recrystallized myosin for a few hours in a larger volume of 0.025 M KCl, then subjected it to the carbonate treatment. The myosin thus obtained dephosphorylated and deaminated ADP no more but did so after the addition of watery muscle extract provided that actin was present. In absence of actin, ADP was dephosphorylated only after a long incubation period. Possibly, in the absence of actin, myosin is unable to bind the protin.

To prepare myosin for the ATP-ase test, Banga dissolved the recrystallized myosin, which had been put through the carbonate treatment, in 0.5–1.0 M KCl, containing 0.1 M acetate buffer of pH 6, and allowed the solution to stand for 1–2 days at room temperature. Her solutions contained 1 mg. of myosin per ml. The myosin was separated by precipitating it through

dilution with eight volumes of water. Banga has also recently used myosin prepared by the ammonium sulphate method and found this myosin distinctly superior to that prepared by the earlier procedure.

The myosin thus treated showed strongly reduced phospholytic activity with ATP. To judge the result of reactivation, the phospholytic activity must be studied in detail. Not only the quantity of free phosphate must be measured but also the initial rate of dephosphorylation. If only the quantity is measured, it is easy to mistake the action of the ADP-P-protein for that of the ATP-protein.

Whereas in ADP, the P and the N are detached together, they are split off independently from ATP. Testing for dephosphorylation, a relatively large quantity of myosin and little protein (watery extract) must be taken. To test for deamination, relatively little myosin and much protein must be used.

#### *Cerebroside*

To test for the action of the cerebroside, the myosin must contain all proteins but must be free of this lipid. To obtain such a preparation, F. Guba extracted muscle with 0.6 M KCl, instead of with acid phosphate. Similarly to the standard method, he diluted with four volumes of water, allowed the actomyosin to precipitate, and then diluted the liquid with equal volumes of water at 0°. The precipitated myosin was kept in suspension by gentle stirring overnight. The myosin thus obtained did not contract on addition of ADP but did so if the very finely suspended cerebroside of the muscle was added.

#### IV. THE PREPARATION OF ACTIN

Actin present in minced muscle in its fibrous form is linked to myosin. To extract it, the myosin must be eliminated, its unextracted residue denatured, and the actin depolymerized. The myosin is extracted by strong salt solutions, its residue denatured by acetone which, at the same time, may split H bonds, linking actin to the structure. The fibrous actin is depolymerized by OH ions in the absence of salts.

Two methods of preparation have hitherto been used in the study of actin: the original borate method of F. B. Straub, and the same method as modified by F. Guba, remodified by Straub. The two methods do not yield the same actin. Both methods yield an actin containing one Ca atom per UW of actin, but the actin prepared by Straub's original method contains two Mg atoms in addition, while the actin prepared by the modified method contains but fractions of one Mg per UW.

The modified method permits using the same muscle for actin preparation which has been used for the preparation of myosin. This method is the only one now used in the author's and Professor Straub's laboratory; hence, it will be the only one described here.

The muscle is minced and extracted with the acid K phosphate, the suspension diluted with water, filtered, and pressed out through a cloth as described in the preparation of myosin. The filtrate is used for the preparation of myosin, the residue for the preparation of actin. The residue is weighed and suspended in four volumes of 0.4%  $\text{NaHCO}_3$ , stirred for 20 minutes, then filtered and pressed out again. The residue is minced once more on a mincer with small holes (1–2mm.).\*

Every 100 g. of the mince is suspended in 100 ml. of a solution containing 0.05 M  $\text{NaHCO}_3$  and 0.05 M  $\text{Na}_2\text{CO}_3$ . The suspension is stored for ten minutes and then diluted with ten volumes of water containing 0.0005 M  $\text{CaCl}_2$ , stirred for ten minutes and then centrifuged.†

The muscle is suspended in three volumes of acetone, strained, and pressed out. The residue is suspended in its equal weight of acetone, allowed to stand for 20 minutes, strained, and pressed out. Now the residue is suspended once more in its equal weight of acetone containing 5 ml. 0.1 M  $\text{Na}_2\text{CO}_3$  per liter, strained, and pressed out, spread on filter paper, and dried in air for 10–15 hours. The dried muscle may be stored for weeks unchanged in the desiccator. If the muscle contained much fat, the extracted actin may be turbid, owing

\*If the myosin was extracted with KCl or the alkaline KCl of Weber then it may be washed with water instead of  $\text{NaHCO}_3$ .

†The  $\text{CaCl}_2$  considerably increases the later yield of actin (Straub).

to the presence of lipoids. This turbidity may be prevented by extracting the muscle with  $\text{Na}_2\text{CO}_3$ -acetone a fourth time.

In the author's laboratory, this method is used in a slightly modified form (Guba). The muscle, extracted with  $\text{NaHCO}_3$  and minced a second time, is not suspended in  $\text{Na}_2\text{CO}_3$ - $\text{NaHCO}_3$  but placed in the refrigerator at  $-12^\circ\text{C}$  overnight. (The muscle may be kept here for any length of time.) When thawed, it is treated with acetone as described above.

The dried muscle is ground in a coffee mill and is then extracted with  $\text{CO}_2$ -free water, 20 ml. being used for every g. of muscle. The powder is mixed with the water and allowed to stand for 10–15 minutes,\* poured on a Buchner funnel, and the solution sucked off. The extract is limpid and contains the actin in globular form in high purity, usually 10 mg. per ml.†

#### *Further Purification*

Nine volumes of acetone, added to the globular actin solution, does not precipitate the protein. On addition of 0.0025 ml. of M pH 4.6 acetate-buffer per ml, actin precipitates in globular form (Straub). The actin may be precipitated from its watery solution at  $0^\circ$  at its IP by acetate. A very dilute buffer must be used, since actin at its IP is very sensitive to ions (Straub). The precipitate is formed instantaneously if the actin solution was not too dilute and is separated rapidly with the centrifuge at  $0^\circ$  and redissolved at once by addition of  $\text{NaHCO}_3$ . It contains the actin partly polymerized. Actin may also be precipitated by small concentrations of  $\text{CaCl}_2$  and redissolved in oxalate.

The primary solution of actin usually contains traces of protins. Protin-free actin can be obtained if the muscle, before acetone treatment, is repeatedly washed with stirring, using great volumes of water.

\*Mixing increases the yield but may make the solution cloudy and prone to spontaneous polymerization.

†The extract should not color phenolphthalein. If it does, mincing was not sufficiently fine and the removal of alkali was incomplete. Alkali destroys actin in solution.

## V. ACTOMYOSIN THREADS

H. H. Weber has discovered that "myosin," dissolved in strong salt solution and squirted in a thin jet into water, forms threads. Myosin forms no threads, and what Weber actually used was actomyosin, or myosin denaturated by copper.

If one simply wants to observe the contractility of actomyosin, freshly minced muscle is extracted with the alkaline KCl of Weber (0.6 M KCl, 0.04 M  $\text{NaHCO}_3$ , and 0.01 M  $\text{Na}_2\text{CO}_3$ ) for 24 hours at  $0^\circ$ , three volumes of fluid being used for every g. of muscle. The actomyosin thus extracted is a highly viscous fluid. Usually, one must dilute it with 0.6 M KCl to permit separation of the undissolved muscle particles on the centrifuge. After centrifugation, the sticky fluid is sucked into a glass tube, one end of which is mounted with a rubber tube, while the other end is drawn out to a gradually narrowed capillary. By breaking off more and more of the point, one finds the proper diameter. A basin is filled with 0.05 M KCl and the capillary end of the tube is dipped into the fluid. The tube is moved right and left as we blow into the rubber tube. With a little practice, fairly uniform threads can be pulled. Threads of about 0.2 mm. diameter are the most convenient. Thinner ones easily curl up, thicker ones are rather sluggish. For the demonstration of contraction, small pieces of 2–3 mm. length are cut out and observed on a hollow ground microscopic slide under the microscope mounted with a low-power lens and an ocular micrometer scale.

A small home-made celluloid spatula of 2–3 mm. diameter can be used with advantage. If this is put under the thread and lifted to some extent, a 2–3 mm. wide piece of the thread may be cut out by striking with a sharp instrument over the edge of the spatula. The thread does not stick to celluloid and, lying upon it, may be transferred into the fluid in which the contraction is to be observed. Contraction is very active in a 0.5–0.1 M KCl, containing 0.0001 M  $\text{MgCl}_2$ . Contraction occurs on addition of 0.1% of ATP. The ATP should reach the thread simultaneously from all sides; otherwise the thread curls up. The author used to squirt the ATP into the suspension fluid under strong pressure from a small pipette with

a capillary end. Blowing on the surface of the fluid from this pipette, after the ATP has been squirted in, might serve for mixing. Solutions of 2% actomyosin are the most suited for preparing threads. Threads prepared from thinner solution may be too fragile, those prepared from more concentrated solution too sluggish.\*

For quantitative studies, more uniform threads should be used, prepared mechanically. Apparatus for mechanical preparation of threads has been described by M. Gerendás [13] and M. and A. Dubuisson.†

\**Arch. Internat. Physiol.* 53, 29, 1943.

†Difficulty may be experienced with very dilute actomyosin solutions or actomyosin of very low actin content. Neutralization of the alkaline solution or the use of 25% glycerol instead of water may facilitate the preparation of threads.